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*Published in:*  
Journal of Clinical Microbiology

*DOI:*  
[10.1128/JCM.02160-07](https://doi.org/10.1128/JCM.02160-07)

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2008

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Esposito, S., Bosis, S., Niesters, H. G. M., Tremolati, E., Sabatini, C., Porta, A., Fossali, E., Osterhaus, A. D. M. E., & Principi, N. (2008). Impact of human bocavirus on children and their families. *Journal of Clinical Microbiology*, 46(4), 1337-42. <https://doi.org/10.1128/JCM.02160-07>

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## Impact of Human Bocavirus on Children and Their Families<sup>†</sup>

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Received 7 November 2007/Returned for modification 11 January 2008/Accepted 9 February 2008

**This study was planned to investigate the prevalence and clinical features of the illnesses associated with human bocavirus (hBoV) in children with acute disease. We prospectively enrolled all subjects aged less than 15 years attending an emergency room in Milan, Italy, on Wednesdays and Sundays between 1 November 2004 and 31 March 2005 for any acute medical reason, excluding surgical diseases and trauma. Nasopharyngeal swabs were collected at admission to detect hBoV; influenza A and B viruses; respiratory syncytial virus; human metapneumovirus; parainfluenza viruses 1, 2, 3, and 4; rhinovirus; adenovirus; and coronaviruses 229E, OC43, NL63, and HKU1 by real-time PCR. Among the 1,332 enrolled children, hBoV was the fifth most frequently detected virus (7.4%). The rate of hBoV coinfections with other viruses was significantly higher than for the other viruses (50.5% versus 27.5%;  $P < 0.0001$ ). Eighty-nine of the 99 hBoV-positive children (89.9%) had a respiratory tract infection, and 10 (10.1%) had gastroenteritis. hBoV coinfections had a significantly greater clinical and socioeconomic impact on the infected children and their households than hBoV infection alone. In conclusion, these findings show that the role of hBoV infection alone seems marginal in children attending an emergency room for acute disease; its clinical and socioeconomic importance becomes relevant only when it is associated with other viruses.**

Two years ago, a previously unknown human parvovirus called human bocavirus (hBoV) was identified in Swedish children suffering from respiratory tract infection (RTI) (2), and subsequent studies carried out in different geographic areas have shown that it circulates widely and can be found in a significant percentage of subjects with upper or lower respiratory tract disease, mainly young children (1, 4–7, 10, 11, 16, 17, 19–22, 24, 25, 28, 30, 32, 33, 39). Healthy carriers of this virus have not been identified. Thus, this strongly supports the hypothesis that hBoV may really be the cause of the diagnosed disease when it is detected in respiratory secretions (3, 26).

Epidemiological studies seem to indicate that, in young children, hBoV is less important than respiratory syncytial virus (RSV) but at least as important as influenza viruses, human metapneumovirus (hMPV), adenovirus, and parainfluenza virus type 3 (1, 4, 11, 16, 19, 25). However, the full spectrum of the diseases associated with hBoV, the prevalence of complications, the frequency of the spread of hBoV in households and close contacts, and hBoV's social consequences are not known. Moreover, the role of coinfections with hBoV and other respiratory viruses remains to be clarified. This information is essential for establishing the real impact of hBoV infections in pediatrics and deciding whether specific preventive or therapeutic measures need to be developed.

The aim of this study was to investigate the prevalence and

clinical features of the illnesses associated with hBoV infections in children attending an emergency room.

### MATERIALS AND METHODS

**Study design.** We prospectively enrolled all subjects aged less than 15 years attending the emergency room of the Institute of Pediatrics, University of Milan, Milan, Italy, on Wednesdays and Sundays between 1 November 2004 and 31 March 2005 for any acute medical reason, excluding surgical diseases and trauma. The study protocol was approved by the Institutional Review Board of the University of Milan, Milan, Italy. The written informed consent of a parent or legal guardian was required, and older children were asked for their assent.

**Patient enrollment and evaluation.** Upon enrollment, systematic records were made of the patients' demographic characteristics and medical history using standardized written questionnaires (8, 12, 13). The questions included detailed disease signs and symptoms, laboratory tests and/or radiological examinations, prescribed drug therapy, chronic underlying diseases, family size and the number of siblings, parents' education and occupations, family living conditions, and information about child care attendance. After a complete physical examination, the children were divided into different disease groups on the basis of signs and symptoms, using well-established criteria (14). Fever was defined as the presence of an axillary temperature of  $\geq 37.8^{\circ}\text{C}$  or a rectal temperature of  $\geq 38^{\circ}\text{C}$  (14).

Upon enrollment, Virocult (Medical Wire and Equipment, Corsham, United Kingdom) nasopharyngeal swabs were used to collect specimens for the detection of hBoV; influenza A and B viruses; RSV; hMPV; parainfluenza viruses 1, 2, 3, and 4; rhinovirus; adenovirus; and human coronaviruses (hCoVs) 229E, OC43, NL63, and HKU1 by real-time PCR. Only one sample was taken from each child, from which two aliquots were immediately obtained before they were frozen at  $-80^{\circ}\text{C}$  for analysis at the Department of Virology, Erasmus Medical Center, Rotterdam, The Netherlands. Total nucleic acids were routinely isolated on a MagnaPureLC isolation station (Roche Applied Science, Penzberg, Germany). A universal internal control virus was used to monitor the process from the isolation of nucleic acids until real-time detection (9, 12). The in-house real-time PCR for hBoV and the internal control, phocine distemper virus, was designed using primer express software (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). hBoV was detected in extracted DNA by PCR amplification of a 291-bp fragment of the NS1 gene, as previously described (1, 27). Briefly, nucleic acids were extracted from 400  $\mu\text{l}$  of the nasopharyngeal aspirates, and the DNA was eluted in 100  $\mu\text{l}$  of RNase-free water. The 20- $\mu\text{l}$  amplification reaction mixture contained 5  $\mu\text{l}$  sample DNA, 10  $\mu\text{l}$  TaqMan universal PCR

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<sup>†</sup> Published ahead of print on 20 February 2008.

TABLE 1. Frequency of hBoV infections

Virus detection	No. (%) of samples that were hBoV	
	Positive <sup>a</sup>	Negative
Infection with a single virus	49 (49.5)*	475 (72.5)
Coinfection with:	50 (50.5)*	180 (27.5)
One other virus	41 (41.4)**	180 (27.5)
Two other viruses	9 (9.1)**	0 (0.0)
Total	99 (100.0)	655 (100.0)

<sup>a</sup>  $P$  was <0.0001 (\*) and  $P$  was <0.05 (\*\*) versus children with hBoV-negative samples. There was no other significant difference between the groups.

master mix (PE Applied Biosystems), 0.1  $\mu$ l bovine serum albumin (20 mg/ml), 300 nmol/liter of each primer (Boca-forward, GGA AGA GAC ACT GGC AGA CAA; Boca-reverse, GGG TGT TCC TGA TAT GAG C), and 150 nmol/liter of the Boca probe (FAM-CTG CGG CTC CTG CTC CTG TGA T-TAMRA, where FAM is 6-carboxyfluorescein and TAMRA is 6-carboxytetramethylrhodamine). Amplification was performed using an ABI 7500 instrument under standard amplification conditions with the following settings: 95°C for 10 min and 42 cycles of 95°C for 15 s and 60°C for 1 min.

Real-time PCR for influenza A and B viruses; RSV; hMPV; parainfluenza viruses 1, 2, 3, and 4; rhinovirus; adenovirus; and hCoVs 229E, OC43, NL63, and HKU1 was performed as previously described (8, 9, 12, 13, 15, 18, 23, 34–38, 40, 41).

The medical histories of the children were reevaluated 5 to 7 days after enrollment and until the resolution of their illness by means of interviews and clinical examinations by trained investigators using standardized questionnaires (8, 12, 13). During this evaluation, information was also obtained regarding acute illnesses and related morbidity in their households. The children's parents or legal guardians were asked to answer questions regarding the outcome of their child's disease (e.g., final diagnosis, administered medication, hospitalization, duration of signs/symptoms, medical visits, examinations, and number of lost school days) and the involvement of other family members (e.g., diseases in households, medication, hospitalization, medical visits, number of working days lost by parents to care for themselves and their children, and the number of domestic help days required to care for the ill children). All the data were verified from medical records.

**Statistical analysis.** The data were analyzed using SAS Windows v.12 (SAS Institute, Cary, NC); a  $P$  value of <0.05 was considered statistically significant. Parametric data were compared by analysis of variance; abnormally distributed or nonparametric data were analyzed using the Kruskal-Wallis test. The categorical data were analyzed by means of contingency analysis and the chi-square or Fisher test.

## RESULTS

**Study population.** We enrolled 1,332 children aged less than 15 years (718 [53.9%] were male; mean age  $\pm$  standard deviation,  $3.32 \pm 3.26$  years); 779 (58.5%) had evidence of acute RTI, 203 (15.2%) had gastrointestinal disease, 86 (6.4%) had fever without source, 49 (3.7%) had exanthematic disease, 41 (3.1%) had seizures with or without fever, 39 (2.9%) had nephritic or nephrotic syndrome, 36 (2.7%) had skin and soft tissue infections, 33 (2.5%) had meningitis/encephalitis, 31 (2.3%) had sepsis, 27 (2.0%) had bone or joint infection, 5 (0.4%) had a coagulation disorder, and 3 (0.3%) had conjunctivitis.

Of the study children, 101 (7.6%) had evidence of chronic underlying diseases: 60 (4.5%) had chronic asthma, 18 (1.3%) had cystic fibrosis, 12 (0.9%) had hemodynamically significant cardiac disease, 6 (0.5%) had chronic kidney insufficiency, and 5 (0.4%) had human immunodeficiency virus (HIV) infection.

**Frequency of hBoV infection.** hBoV was the fifth most frequently detected virus and was identified in 99 children (7.4%). The most frequently detected viruses were influenza viruses (180 cases, 13.5%), whereas RSV was found in 161 patients (12.1%), adenovirus in 123 (9.2%), rhinovirus in 101 (7.6%), hCoVs in 53 (4.0%), parainfluenza viruses in 35 (2.6%), and hMPV in 2 (0.2%).

Coinfections were demonstrated in 230/1,332 children (17.3%). hBoV was the most frequent virus in coinfections, being demonstrated together with at least one other respiratory virus in 50/99 hBoV-positive samples (50.5%; there were 13 subjects with adenovirus, 12 with influenza viruses, 8 with RSV, 3 with CoVs, 3 with rhinovirus, 2 with parainfluenza viruses, and 9 with multiple viruses). Coinfections in the presence of viruses different from hBoV were observed in 180/655 cases (27.5%). The rate of coinfections with hBoV and other viruses was significantly higher than those for any combination of other respiratory viruses ( $P < 0.0001$ ) (Table 1).

hBoV was detected in 89/779 children with RTIs (11.4%) and 10/203 children with gastrointestinal disease (4.9%). No other clinical diagnosis was associated with hBoV.

Table 2 shows the demographic characteristics of the chil-

TABLE 2. Demographic characteristics in children with infections caused by a single virus or by hBoV associated with other viruses

Infection	No. of children	No. (%) of males	No. (%) of children of indicated age (yr)			Mean age (yr) (SD)	No. (%) of children with chronic underlying disease
			<2	2–5	>5		
hBoV alone	49	29 (59.2)	36 (73.5) <sup>c,f</sup>	13 (26.5) <sup>b,d,f,g</sup>	0 (0.0) <sup>a,c,e,f,h</sup>	2.4 (2.5) <sup>a,b,e,f</sup>	0 (0.0) <sup>a</sup>
hBoV coinfection	50	29 (58.0)	28 (56.0) <sup>b,f</sup>	11 (22.0) <sup>b,d,f,g</sup>	11 (22.0) <sup>d,g</sup>	3.1 (2.6)	8 (16.0)
Influenza virus	151	76 (50.3)	46 (30.5)	66 (43.7)	39 (25.8) <sup>d,g</sup>	3.6 (2.9)	10 (6.7)
RSV	121	68 (56.2)	61 (50.4) <sup>b,f</sup>	51 (42.1)	9 (7.4) <sup>h</sup>	1.9 (1.8) <sup>a,c,e,f</sup>	9 (7.4)
Rhinovirus	74	39 (52.7)	39 (52.7) <sup>b,f</sup>	21 (28.4)	14 (18.9)	3.0 (2.9)	5 (6.8)
Adenovirus	70	41 (58.6)	39 (55.8) <sup>b,f</sup>	26 (37.1)	5 (7.1) <sup>h</sup>	2.3 (1.9) <sup>a,b,e,f</sup>	2 (2.9)
hCoVs	36	21 (58.3)	11 (30.6) <sup>b</sup>	15 (41.6)	10 (27.8)	3.6 (2.7)	3 (8.3)
Parainfluenza virus	21	12 (57.1)	12 (57.1) <sup>f</sup>	9 (42.9)	0 (0.0) <sup>h</sup>	1.7 (1.3) <sup>a,b,e,f</sup>	0 (0.0)
hMPV	2	1 (50.0)	0 (0.0)	0 (0.0)	2 (100.0)	6.6 (0.9)	0 (0.0)

<sup>a</sup>  $P < 0.05$  versus children with hBoV coinfection.

<sup>b</sup>  $P < 0.05$  versus children with influenza virus.

<sup>c</sup>  $P < 0.0001$  versus children with influenza virus.

<sup>d</sup>  $P < 0.05$  versus children with RSV.

<sup>e</sup>  $P < 0.05$  versus children with rhinovirus.

<sup>f</sup>  $P < 0.05$  versus children with hCoVs.

<sup>g</sup>  $P < 0.05$  versus children with parainfluenza virus.

<sup>h</sup>  $P < 0.05$  versus children with hMPV. No other significant between-group difference was observed.

TABLE 3. Clinical characteristics of children with infections caused by a single virus or by hBoV associated with other viruses

Diagnosis	No. (%) of children with:								
	hBoV alone ( <i>n</i> = 49)	hBoV coinfection ( <i>n</i> = 50)	Influenza virus ( <i>n</i> = 151)	RSV ( <i>n</i> = 121)	Rhinovirus ( <i>n</i> = 74)	Adenovirus ( <i>n</i> = 70)	hCoVs ( <i>n</i> = 36)	Parainfluenza virus ( <i>n</i> = 21)	hMPV ( <i>n</i> = 2)
URTI	42 (85.7) <sup>a,b,d,e,f,i</sup>	21 (42.0) <sup>b,d,e,g,i</sup>	100 (66.2)	39 (32.2)	46 (62.2)	43 (61.4)	25 (69.4)	16 (76.2)	0 (0.0)
Pharyngitis	27 (55.1) <sup>c</sup>	9 (42.8)	79 (52.3) <sup>c</sup>	27 (22.3)	30 (40.5) <sup>c</sup>	31 (44.3) <sup>c</sup>	24 (66.7) <sup>d</sup>	7 (33.3)	0 (0.0)
AOM <sup>j</sup>	9 (18.4) <sup>f</sup>	7 (14.0) <sup>f</sup>	14 (9.3)	9 (7.4)	10 (13.5)	9 (12.8)	0 (0.0)	5 (23.8)	0 (0.0)
Rhinosinusitis	6 (12.2) <sup>c</sup>	5 (10.0) <sup>c</sup>	7 (4.6)	3 (2.5)	6 (8.2)	3 (4.3)	1 (2.7)	4 (19.1)	0 (0.0)
LRTI	2 (4.0) <sup>a,b,d,e,i</sup>	24 (48.0) <sup>b,e,f,g,h</sup>	35 (23.2) <sup>c</sup>	69 (57.0) <sup>c,f,g,h</sup>	12 (16.2)	4 (5.8)	5 (13.9)	0 (0.0)	2 (100.0)
Acute bronchitis	1 (2.0) <sup>a,b,c</sup>	9 (18.0) <sup>f</sup>	18 (11.9)	14 (11.6)	6 (5.4)	2 (2.9)	2 (5.6)	0 (0.0)	1 (50.0)
Wheezing	1 (2.0) <sup>d</sup>	7 (14.0) <sup>c</sup>	6 (4.0) <sup>d</sup>	39 (32.2)	4 (5.4) <sup>d</sup>	0 (0.0)	1 (2.7) <sup>d</sup>	0 (0.0) <sup>c</sup>	1 (50.0)
Pneumonia	0 (0.0) <sup>a,c</sup>	8 (16.0) <sup>e,f</sup>	11 (7.3)	16 (13.2) <sup>e,f</sup>	2 (2.7)	2 (2.9)	2 (5.6)	0 (0.0)	0 (0.0)
Gastroenteritis	5 (10.2)	5 (10.0)	6 (4.0) <sup>f</sup>	11 (9.1)	8 (10.8)	18 (25.7)	4 (11.1)	3 (14.3)	0 (0.0)
Fever without source	0 (0.0)	0 (0.0)	10 (6.6)	2 (1.7)	2 (2.7)	1 (1.4)	2 (5.6)	2 (9.5)	0 (0.0)
Exanthema	0 (0.0)	0 (0)	0 (0.0)	0 (0.0)	6 (8.1)	4 (5.7)	0 (0.0)	0 (0.0)	0 (0.0)

<sup>a</sup> *P* < 0.05 versus children with hBoV coinfection.<sup>b</sup> *P* < 0.05 versus children with influenza virus.<sup>c</sup> *P* < 0.05 versus children with RSV.<sup>d</sup> *P* < 0.0001 versus children with RSV.<sup>e</sup> *P* < 0.05 versus children with rhinovirus.<sup>f</sup> *P* < 0.05 versus children with adenovirus.<sup>g</sup> *P* < 0.05 versus children with hCoVs.<sup>h</sup> *P* < 0.05 versus children with parainfluenza virus.<sup>i</sup> *P* < 0.05 versus children with hMPV. There was no other significant between-group difference.<sup>j</sup> AOM, acute otitis media.

dren with infections caused by a single virus or by hBoV associated with other viruses. There was no significant between-group difference in gender distribution. The age distribution and mean age of the children with hBoV infection alone were similar to those of the children with RSV, adenovirus, and parainfluenza virus, but their mean age was less than that of the children with hBoV coinfection, influenza, rhinovirus, hCoV, or hMPV. Moreover, the presence of a chronic underlying disease was significantly less frequent in the children with hBoV infection alone than in those with hBoV coinfection. Among children with hBoV coinfection, five had chronic asthma, two had hemodynamically significant cardiac disease, and one had HIV infection; among children with influenza, six had chronic asthma, two had cystic fibrosis, one had hemodynamically significant cardiac disease, and one had chronic kidney insufficiency; among children with RSV, three had chronic asthma, two had cystic fibrosis, two had hemodynamically significant cardiac disease, one had chronic kidney insufficiency, and one had HIV infection; among children with rhinovirus infection, two had chronic asthma, two had cystic fibrosis, and one had hemodynamically significant cardiac disease; among children with adenovirus infection, one had chronic kidney insufficiency and one had HIV infection; among children with hCoVs, two had chronic asthma and one had cystic fibrosis.

**Clinical and socioeconomic relevance of hBoV infection in infected children.** Table 3 summarizes the clinical characteristics of the children with infections caused by a single virus or by hBoV associated with other viruses. Of the 99 hBoV-positive children, 89 (89.9%) had RTIs and 10 (10.1%) gastroenteritis. In the children with hBoV infection alone, upper RTIs (URTIs) were the most frequently diagnosed diseases (85.7%), whereas

lower RTIs (LRTIs) were found in only 4.0%; the opposite was true for children with hBoV coinfection. The prevalence of URTIs in children with hBoV infection alone was higher than that in the children with other viral infections, although influenza virus, rhinovirus, adenovirus, hCoVs, and parainfluenza virus were associated mainly with URTIs. On the other hand, the prevalence of LRTIs was higher in the children with hBoV coinfection or with RSV or hMPV than in the other groups. Interestingly, among the other infections, the prevalence of gastroenteritis was quite high regardless of the etiology of the diseases, and there were similar prevalences of gastroenteritis in children with hBoV infection alone or hBoV coinfection and in children in the other viral groups.

Table 4 summarizes the clinical and socioeconomic impact of the infections caused by a single virus or by hBoV associated with other viruses on the children. The percentages of children requiring laboratory tests and radiographic examinations upon admission and the hospitalization rate were higher for the children with hBoV coinfection than for those with hBoV infection alone or those in the other viral groups, and the median number of lost school days due to the admission illness was also higher for the children with hBoV coinfection. Antibiotics were more frequently prescribed for the children with hBoV infection alone or hBoV coinfection than for children in the other viral groups, whereas the prescription of acetaminophen was significantly more common for the children with influenza. Steroids and aerosol therapy were prescribed more frequently for the children with hBoV coinfection than for those with hBoV infection alone or for those in the majority of the other viral groups.



TABLE 4. Clinical and socioeconomic impact on children with infections caused by a single virus or by hBoV associated with other viruses

Infection (no. of children)	No. (%) requiring:			Median no. (range) who missed a school day	No. (%) prescribed:			
	Laboratory testing	Radiographic examination	Hospitalization		Antibiotics	Acetaminophen	Steroids	Aerosol therapy
hBoV alone (49)	13 (26.5) <sup>a</sup>	2 (4.1) <sup>a,c</sup>	2 (4.1) <sup>a</sup>	4 (1–16) <sup>a</sup>	30 (61.2) <sup>c,d,e,f</sup>	0 (0.0) <sup>b</sup>	1 (2.0) <sup>a,c</sup>	3 (6.1) <sup>a,c</sup>
hBoV coinfection (50)	25 (50.0)	11 (22.0)	10 (20.0)	14 (2–18)	39 (78.0) <sup>c,d,e,f,g</sup>	2 (4.0) <sup>b</sup>	9 (18.0)	15 (30.0)
Influenza virus (151)	35 (23.2) <sup>a</sup>	12 (8.0) <sup>a,c</sup>	7 (4.6) <sup>a,c,e</sup>	9.5 (7–10)	89 (58.9)	121 (80.1)	10 (6.7) <sup>a,c</sup>	9 (6.0) <sup>a,c</sup>
RSV (121)	24 (19.8) <sup>a</sup>	23 (19.0)	19 (15.7)	7 (5–14)	52 (43.0)	56 (46.3) <sup>b</sup>	19 (15.7)	34 (28.1)
Rhinovirus (74)	12 (16.2) <sup>a</sup>	4 (5.4) <sup>a,c</sup>	4 (5.4) <sup>a</sup>	0 (0–2) <sup>a</sup>	24 (32.4)	30 (40.5) <sup>b</sup>	8 (10.8)	5 (6.8) <sup>a,c</sup>
Adenovirus (70)	21 (30.0) <sup>a</sup>	1 (1.4) <sup>a,c</sup>	1 (1.4) <sup>a</sup>	2 (1–6) <sup>a</sup>	26 (37.1)	43 (61.4) <sup>b</sup>	4 (5.7) <sup>a,c</sup>	2 (2.9) <sup>a,c</sup>
hCoVs (36)	8 (22.2) <sup>a</sup>	1 (2.8) <sup>a,c</sup>	3 (8.3)	5 (2–7)	11 (30.6)	43 (61.4) <sup>b</sup>	0 (0.0) <sup>a,c</sup>	3 (8.3)
Parainfluenza virus (21)	3 (14.3) <sup>a</sup>	1 (4.8)	1 (4.8)	0 (0–1) <sup>a</sup>	7 (33.3)	11 (52.4) <sup>b</sup>	0 (0.0) <sup>a,c</sup>	1 (4.8) <sup>a,c</sup>
hMPV (2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0)	30 (61.2) <sup>c,d,e,f</sup>	0 (0.0) <sup>b</sup>	1 (2.0) <sup>a,c</sup>	3 (6.1) <sup>a,c</sup>

<sup>a</sup> *P* < 0.05 versus children with hBoV coinfection.<sup>b</sup> *P* < 0.05 versus children with influenza virus.<sup>c</sup> *P* < 0.05 versus children with RSV.<sup>d</sup> *P* < 0.05 versus children with rhinovirus.<sup>e</sup> *P* < 0.05 versus children with adenovirus.<sup>f</sup> *P* < 0.05 versus children with hCoVs.<sup>g</sup> *P* < 0.05 versus children with parainfluenza virus. There was no other significant between-group difference.

**Clinical and socioeconomic impact of hBoV infection on households.** Table 5 summarizes the clinical and socioeconomic impact on households of children with infections caused by a single virus or by hBoV associated with other viruses. The prevalence of infections similar to that of the study child, the number of medical visits required, the median number of lost working days because of household illnesses, and the median number of lost school days because of sibling illnesses, as well as the number of antibiotic and antipyretic prescriptions, were all higher in the households of children with hBoV coinfection and influenza than in those with hBoV infection alone or those in the other viral groups. There was no significant between-group difference in the household hospitalization rates.

## DISCUSSION

Our findings show that the role of hBoV infection alone seems to be marginal in children attending an emergency room for acute disease and that it becomes important only when it is

associated with other viral pathogens. This conclusion is supported by the epidemiological and clinical data and confirmed by the socioeconomic impact of hBoV infections.

Several recent studies have demonstrated that hBoV is frequently isolated in children and that its presence is almost always associated with URTI and/or LRTI (1, 4–7, 10, 11, 16, 17, 19–22, 24, 25, 28, 30–33, 39). Our data confirm this, as hBoV was detected in 7.4% of the pediatric patients admitted to our emergency room and in 11.4% of those with respiratory problems. The frequency of hBoV infections in our study is in the range of the data reported by other authors, who have detected the virus in 1.5 to 19% of subjects with acute respiratory illness (1, 4–7, 10, 11, 16, 17, 19–22, 24, 25, 28, 30–33, 39), a range that can at least partially be explained by the different methods used to study the epidemiology of hBoV infection. Our study was prospective, and the samples were taken from all of the children admitted to the emergency room for acute diseases, most of whom were sent home because they

TABLE 5. Clinical and socioeconomic impact on households of children with infections caused by a single virus or by hBoV associated with other viruses

Infection (no. of children)	No. (%)		Median no. (range) who missed:		No. (%) requiring:		
	With an infection similar to that of study child	Requiring a medical visit	Work day(s)	School day(s)	Hospitalization	Antibiotic prescription	Antipyretic prescription
hBoV alone (120)	14 (11.7) <sup>c</sup>	9 (7.5) <sup>b</sup>	1 (0–4) <sup>a,b</sup>	1 (0–2) <sup>a,b</sup>	0 (0.0)	4 (3.3) <sup>a</sup>	9 (7.5) <sup>b</sup>
hBoV coinfection (126)	21 (16.7) <sup>c,e</sup>	18 (14.3) <sup>c</sup>	5 (2–7)	3 (1–6)	1 (2.0)	14 (11.1)	20 (15.9)
Influenza virus (400)	71 (17.8) <sup>d,e</sup>	69 (17.2) <sup>d</sup>	5 (2–7)	4 (0–8)	4 (1.0)	36 (9.0)	69 (17.2)
RSV (310)	39 (12.6) <sup>c</sup>	30 (9.7) <sup>b</sup>	2 (1–5)	2 (1–6)	2 (0.6)	18 (5.8)	33 (10.6)
Rhinovirus (190)	9 (4.7)	5 (2.6)	2 (1–5)	2 (1–5)	0 (0.0)	4 (2.1) <sup>a,b</sup>	9 (4.7) <sup>a,b</sup>
Adenovirus (170)	14 (8.2)	12 (7.1) <sup>b</sup>	2 (1–5)	2 (1–6)	0 (0.0)	9 (5.3)	12 (7.1) <sup>b</sup>
HCoVs (90)	9 (10.0)	6 (6.7) <sup>b</sup>	1 (1–3) <sup>a,b</sup>	1 (0–2) <sup>a,b</sup>	0 (0.0)	3 (3.3)	6 (6.7) <sup>b</sup>
Parainfluenza virus (55)	4 (7.3)	3 (5.5) <sup>b</sup>	2 (1–3)	0 (0) <sup>a,b</sup>	0 (0.0)	2 (3.6)	3 (5.5) <sup>b</sup>
HMPV (6)	0 (0.0)	0 (0.0)	0 (0)	0 (0)	0 (0.0)	0 (0.0)	0 (0.0)

<sup>a</sup> *P* < 0.05 versus children with hBoV coinfection.<sup>b</sup> *P* < 0.05 versus children with influenza virus.<sup>c</sup> *P* < 0.05 versus children with rhinovirus.<sup>d</sup> *P* < 0.0001 versus children with rhinovirus.<sup>e</sup> *P* < 0.05 versus children with adenovirus. There was no other significant difference between the different etiologic groups.

were affected by mild infection and some had no infection at all. Most of the other studies included only hospitalized children or children with LRTIs (1, 4–7, 10, 11, 16, 17, 19, 20, 22, 24, 25, 28, 30–33, 39). As hBoV infection alone seemed to cause mainly mild diseases in our population, it is reasonable to think that the incidence of hBoV infections is lower when only severe diseases are considered.

About half of the respiratory samples in which hBoV was identified came from children who were also infected by at least one of the other respiratory viruses tested. A high incidence of hBoV coinfections in children with respiratory diseases has been reported in previous studies (1, 4, 11, 17, 25, 39), but their clinical significance is not clear. We found that the frequency of hBoV coinfections was greater than that of coinfections involving the other viruses. Furthermore, hBoV infection alone was usually mild and the hBoV coinfections were significantly more severe. The relatively benign nature of hBoV is highlighted by the fact that the mean age of the children with hBoV infection alone was significantly lower than that of the children with hBoV coinfection and similar to that of children with RSV; however, both hBoV coinfection and RSV were more severe in clinical and socioeconomic terms.

Our data provide further information concerning the total burden of hBoV infection. They not only demonstrate that hBoV coinfections can be observed in children with chronic underlying diseases, which underlines the importance of further studies of the role of hBoV in chronically ill pediatric patients, but also describe the clinical characteristics of hBoV infection in more detail by revealing significant differences in the presentations of hBoV infection alone and hBoV coinfection, as well as between hBoV infection alone and infections due to other viruses. Furthermore, the fact that signs and symptoms of acute gastroenteritis were the reason for attending the emergency room for about 10% of the children in whom hBoV was detected offers a clinical explanation for the recent demonstration of hBoV DNA in stool samples (29).

Finally, our data indicate that hBoV infection alone has a marginal clinical and socioeconomic impact on infected children, whereas hBoV coinfection seems to be associated with higher direct and indirect costs, as it involves significantly more examinations, hospitalization, lost school days, and different drug therapies. In addition, and in line with the clinical data, an hBoV coinfection increases direct and indirect family costs.

In conclusion, our data indicate that hBoV is a common respiratory virus and suggest that, alone, it is associated mainly with mild respiratory or gastrointestinal illnesses, but its clinical and socioeconomic impact on infected children and their families becomes significant when it is present in coinfections with other viruses. Although further data are needed to confirm these findings, the systematic surveillance of hBoV infection in children with acute disease does not seem to be a priority.

#### ACKNOWLEDGMENT

This study was supported in part by a grant from the Italian Ministry of Universities (project no. 2005068289\_001).

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